

English version of the final report

Final Report

An Acute Dermal Irritation/Corrosion Study of in Rabbits

Study No. H-10234

February 5, 2011

Nippon Experimental Medical Research Institute Co., Ltd.
1967-11 Arima, Shibukawa-shi, Gunma, Japan

STATEMENT OF COMPLIANCE

Title : An Acute Dermal Irritation/Corrosion Study of in Rabbits

Study No. H-10234

I, the undersigned, hereby declare that this report is the English version of the original report that has been written in Japanese language. Further, I declare that the data are exactly reflected in this report and similar to that of the original (Japanese) report.

Takashi Sato

Date: June 11, 2012

Takashi Sato, M.S.

Study Director (translator)

Nippon Experimental Medical Research Institute Co., Ltd.

PREPARATION OF THE FINAL REPORT

Title: An Acute Dermal Irritation/Corrosion Study of in Rabbits
Study No.: H-10234

The study listed above was performed in compliance with the Organization for Economic Co-operation and Development (OECD) Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17.

This study was conducted according to the methods described herein, and the data are accurately reflected in this report.

Study director : Takashi Sato, M.S. (Impression of the seal)

Date : February 5, 2011

Nippon Experimental Medical Research Institute Co., Ltd.

OUTLINE OF THE STUDY CONDUCT

Title: An Acute Dermal Irritation/Corrosion Study of in Rabbits
Study No. :H-10234

1. Purpose

As part of the safety assessment, was investigated for dermal irritation/corrosion in rabbits.

2. GLP compliance

Organisation for Economic Co-operation and Development(OECD)Principles of Good Laboratory Practice (as revised in 1997), ENV/MC/CHEM(98)17

3. Guidelines referred

This study was conducted by reference to the OECD Guideline for the Testing of Chemicals #404 (adopted on April 24, 2002) (hereinafter referred to as “OECD Guideline”).

4. Animal welfare

This study was approved by the Animal Experimental Committee of Nippon Experimental Medical Research Institute Co., Ltd. (Approval No. 2006013).

5. Sponsor

Name:

Address:

6. Contract laboratory

Name: Nippon Experimental Medical Research Institute Co., Ltd.

Address: 1967-11 Arima, Shibukawa-shi, Gunma, Japan

7. Testing facility

Name: Shibukawa Laboratories

 Nippon Experimental Medical Research Institute Co., Ltd.6.

Address: 1967-11 Arima, Shibukawa-shi, Gunma, Japan

8. Storage of records and materials

(1) Storage period

The items are stored for 10 years after completion of the study.

(2) Storage items and storing location

The study protocol and its amendment (original), documents relating to the study, raw data obtained in the course of the study, final report (original) and other materials are stored in Shibukawa Laboratories, Nippon Experimental Medical Research Institute Co., Ltd.

(3) Address of the storing institute

1967-11 Arima, Shibukawa-shi, Gunma, Japan

9. Schedule of the study

Study initiation	: December 28, 2010
Animal arrival	: January 6, 2011
End of the quarantine period	: January 11, 2011
Start of the experiment	: January 12, 2011
First administration	: January 12, 2011
Observation	: January 12, 2011
Completion of the experiment	: January 12, 2011
Submission of the draft final report	: January 24, 2011
Preparation of the final report	: February 5, 2011
Completion of the study	: February 5, 2011

10. Study personnel and work responsibility

Study director, protocol preparation, work supervising/management and preparation of the final report	: Takashi Sato ^{Note)}
Clinical observation and body weight measurement during the quarantine/acclimation period	: Masaaki Shirai, Yoko Wakana, Moeko Masuyama, Takashi Sato
Animal health assessment	: Yoko Wakana
Preparation of the test substance	: Takashi Sato
Control of the test substance	: Takashi Sato
Administration, removal, washing, clinical observation and body weight measurement	: Masaaki Shirai, Yoko Wakana, Takashi Sato
Observation of treatment sites	: Yoko Wakana, Takashi Sato

Note) Affiliation: Safety Research Department, Nippon Experimental Medical Research
Institute Co., Ltd.

Address: 1967-11 Arima, Shibukawa-shi, Gunma, Japan



UNPREDICTED EVENTS CONSIDERED TO HAVE AFFECTED THE RELIABILITY OF THE STUDY AND DEVIATION FROM THE PROTOCOL

Title: An Acute Dermal Irritation/Corrosion Study of in Rabbits
Study No.: H-10234

1. Unpredicted events

The humidity in Animal Room 8 deviated from the lower limit between 9:54:40 and 9:54:47 a.m., and between 9:54:56 a.m. and 10:16:28 a.m. on January 7, 2011, recording the lowest value of 29.9% and 22.6%, respectively. The deviations occurred due to vapor-supply disruption caused by an error of the maintainer shutting the vapor valve of the boiler.

2. Deviation from the protocol

There were no relevant items.

3. Effects on the reliability of the study

The deviations of the humidity were considered to have no effect on the reliability of the study, because no abnormal finding was observed in general conditions of animals after the humidity returned to the normal range.

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I. Summary

was investigated for acute dermal irritation/corrosion in male Japanese White rabbits.

Three application sites were prepared in the clipped dorsal skin of one rabbit. A 0.5g of the test substance was spread on each of 2.5×2.5 -cm lint patches, and occlusively applied to each application site. First, a patch was removed after application for 3min, and the topical site was observed for the presence or absence of corrosion. Since no corrosion was noted in the application site just after removal of the patch, the animal was subjected to occlusive exposure for 1hr. As a result, corrosion was noted just after removal; therefore, no more observation was performed.

Based on the above results, it was concluded that was a corrosive substance under the conditions of this study.

II. Purpose of study

As part of safety assessment, was investigated for acute dermal irritation/corrosion in rabbits.

III. Materials and methods

1. Test substance ^{Note 1)}

- (1) Name (abbr. name) :
- (2) Chemical name :
- (3) Rational formula :
- (4) Molecular formula :
- (5) Molecular weight :
- (6) CAS No. :
- (7) Lot No. :
- (8) Purity :
- (9) Property at ambient temperature
:
- (10) Name of impurities and the concentration
: Unknown, 0.5%
- (11) Storing conditions : Light shielding at room temperature

Note 1: Characteristics and the stability were based on information (Non-GLP) from the sponsor

(12) Expiration date : May 27, 2011

(13) Solubility : Soluble in water, dimethyl sulfoxide (DMSO) and acetone

(14) Identity (SOP 13-01-05) (GLP)

: In the study (Study No. H-10233) using the same test substance, before the start of experiment, the infrared absorption spectrum of the test substance was measured with an infrared spectrophotometer (Fourier transform infrared spectrophotometer, FT-720, HORIBA, Ltd) (December 28, 2010). Comparing with the infrared absorption spectrum of the lot provided from the sponsor, confirmed the identity of the test substance based on the similarity of main peak shapes.

(15) Source

Name :

Address :

(16) Handling of the residual test substance

:Residual test substance is returned to the department given below after the end of the relevant experiments.

Name

Address

2. Preparation of the test substance

The test substance was not diluted and used as supplied.

3. Experimental animals

Rabbits were chosen according to the OECD Guideline, especially Japanese White rabbits because they were generally used in acute dermal irritation/corrosion studies. Four 10-week old male Japanese White rabbits (Kbs:JW, Healthy) (body weight range: 1.90-1.96kg) produced by Kitayama Labes Co., Ltd. (3052-1 Arai, Ina-shi, Nagano, Japan) were purchased from Oriental Bioservice Kanto, Inc. (3-15-15 Azuma, Tsukuba-shi, Ibaraki, Japan). The animals were quarantined during the 6 days following their arrival. During this period, the animals were weighed with a Sartorius electronic balance (E12000S, Sartorius Co., Ltd.) on the first and last days,

observed for clinical signs once every day, and assessed for health conditions on the last day of the period. No abnormal finding was observed throughout the quarantine period, indicating good health conditions. Animals except for the animal used for the first administration were continuously acclimated to housing conditions after the quarantine period, and observed for general conditions.

At approximately 24hr before the first administration, the animals' dorsal hair was removed with an electric hair-clipper (2.0- and 0.5-mm edges, DAITO ELECTRIC MACHINE INDUSTRY CO., LTD.) and pre-treatment skin conditions were examined. Based on the results, 3 healthy animals with good skin conditions were selected for the study, and the other animal which had island skins was reserved as one of pooled animals. Since the experiment was terminated at first administration, used was only one animal. The animal's age at the time of administration was 10 weeks and the body weight was 2.13kg.

Cages were identified with color labels (showing the Study no., name of the test substance, type of the study, sex and temporary animal no. or animal no., etc), and animals with animal nos. written inside of the auricle (left auricle during the quarantine/acclimation period and right auricle after grouping) with oil ink.

4. Housing and environmental conditions

(1) Environmental conditions

The animals were individually housed in automatic water-washing aluminum cages for rabbits (350W × 480D × 350H mm) in a conventional system animal room (Room No.8 during the quarantine period, Room No.9 after the quarantine period), where the environmental conditions were maintained to a temperature of 20±3°C (actual values: Room No.8: 18.4-22.4°C, Room No.9: 19.6-20.1°C), humidity of 50±20% (actual values: Room No.8: 22.6-65.1%, Room No.9: 49.5-58.3%), ventilation frequency of 10 times or more per hour (all-fresh-air system), and lighting of 12hr per day (from 7:00 a.m. to 7:00 p.m., 150-300 lux).

Cages and feeders were sterilized with 500-fold aqueous dilution of sodium hypochlorite prior to use, and changed for new ones at the time of changing the animal room. The animal room was cleaned every day after work and the floor was sterilized by wiping with 200-fold aqueous dilution of benzethonium chloride (Hyamine solution; Daiichi Sankyo Co., Ltd.).

(2) Feed

Pellet diet for experimental animals CR-3 (Lot No. R3070 and, R3080 Clea Japan, Inc., 1-2-7 Higashiyama, Meguro-ku, Tokyo, Japan) was provided *ad libitum* via

feeders. Feed analysis for impurities and contaminants in the lot used in the study was entrusted by the maker to Tokyo Kenbikyo-in foundation (44-1 Nihonbashi Hakozaiki-cho, Chuo-ku, Tokyo, Japan). Based on the results (obtained as a copy), the conformity to the standards specified by Nippon Experimental Medical Research Institute Co., Ltd. was confirmed before feeding.

(3) Drinking water

Shibukawa-city tap water was provided *ad libitum* via an automatic watering system. Water analysis in the samples periodically collected at sites specified by this facility was entrusted to Environmental Technical Co., Ltd. (1709-1 Kaneko-cho, Takasaki-shi, Gunma, Japan) based on the “Ministerial Ordinance Concerning Water Quality Standards (MHLW Ordinance No. 101, 2003)”, “Partial Amendment of Ministerial Ordinance Concerning Water Quality Standards (MHLW Ordinance No. 174, 2008)”, and “Partial Amendment of the Ordinance for Enforcement of the Water Law (MHLW Ordinance No.175, 2008)”. Based on the results, the conformity to the above-mentioned water quality standards was confirmed.

5. Number of animals, dose level and animal numbers

(1) Number of animals, dose level and animal numbers

Number of animals	Dose level	Animal No.
1	0.5g/treatment site	1

(2) Justification for selection of dose level and number of animals.

The dose level and number of animals were according to the OECD Guideline.

6. Administration

(1) Route of administration

Percutaneous route (occlusive patch)

(2) Administration method

Hair-clipped areas (approximately 2.5×2.5 cm, approximately 6 cm² each) of the dorsal region served as application sites. A 0.5g of the test substance was evenly spread on a 2.5×2.5 -cm lint patch (OSATO EISEI ZAIRYO SEIZOSHO, Y.K.) and applied to each application site to contact to the skin. The lint patch was occluded with adhesive elastic bandage cut in 3.5×3.5 cm (Silky Tex, ALCARE CO., LTD.) and fixed with Silky Tex and surgical tape (NICHIBAN CO., LTD.) wrapped around the trunk.

① First administration (Figure 1)

Three application sites (A to C: Figure 1) were prepared on 1 animal (Animal No.1). Firstly, a patch of the test substance was applied to Site A (not wrapped with dressing around the trunk). The patch was removed after an exposure period of 3min and the topical site was observed for corrosion. Because of the absence of corrosion, another patch was applied to Site B. The patch was removed after an exposure period of 1hr and the topical site was observed for corrosion. Because of the presence of corrosion in Site B, the experiment was terminated and further process was not performed. At the time of removing each patch, the test substance remaining on the application sites was removed by gently wiping with absorbent cotton (J.P., KAWAMOTO CORPORATION) moistened with water for injection (J.P., Lot No.00610D, Fuso Pharmaceutical Industries, Ltd.).

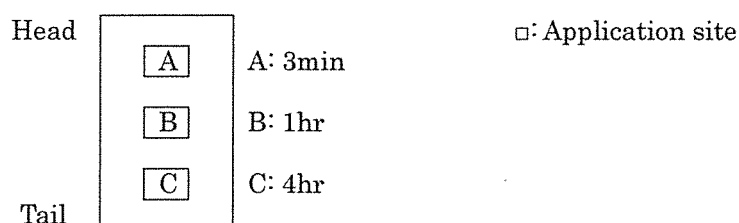


Figure 1. Application sites and duration

② Administration for confirmation

Since corrosion was observed at observation in the first administration, further administration for confirmation was not performed.

(3) Rationale for administration route and method

The administration route and method were in accordance with the OECD Guideline.

7. Observation and measurement

(1) Observation and scoring for dermal irritation/corrosion

Since corrosion was observed at observation in the first administration, scoring was not performed.

(2) Evaluation criteria for dermal reactions

① Erythema and eschar formation	score
No erythema.....	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation preventing grading erythema	4
Maximum score: 4	

② Edema formation	score
No edema.....	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising).....	2
Moderate edema (raised approximately 1 mm).....	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4
Maximum score: 4	

(3) Calculation of P.C.I.

Since corrosion was observed in the first administration, P.C.I was not calculated.

(4) A.F.N.O.R. standards for dermal irritation intensity

$P.C.I. < 0.5$	Non irritant
$0.5 \leq P.C.I. \leq 3$	Slightly irritant
$3 < P.C.I. \leq 5$	Moderately irritant
$5 < P.C.I. \leq 8$	Severely irritant

(5) Clinical observation and body weight measurement

One treated animal was observed for clinical signs, and weighed on the day of administration (Day 0) with a Sartorius electronic balance (E12000S, Sartorius Co., Ltd.).

(6) Handling of the residual animals

After the end of observation, the used animals were reserved as pooled animals.

8. Statistical analysis

The mean values were calculated for the scores and values obtained in this study; however statistical analyses were not performed.

IV. Results

1. Evaluation of dermal irritation/corrosion (Table1, Appendix 1)

In the 3min-treatment site in the first administration, very slight erythema was noted at observation just after removal of the patch; however, no necrosis or corrosive reaction was observed. In the 1hr-treatment site, well defined erythema, and edema raised 1mm or more and extending beyond the area of exposure, as well as corrosion, were noted at observation just after removal of the patch,.

2. Clinical observation (Table 2)

No abnormal finding was observed during the observation period.

3. Body weight measurement (Table 3)

The body weight at the time of administration was 2.13kg.

V. Discussion and conclusion

was investigated for acute dermal irritation/corrosion in male Japanese White rabbits.

Three application sites were prepared in the clipped dorsal skin of one rabbit. A 0.5g of the test substance was spread on each of 2.5×2.5 -cm lint patches, and occlusively applied to each application site. First, a patch was removed after application for 3min, and the topical site was observed for the presence or absence of corrosion. Since no corrosion was observed in the application site just after removal of the patch, the animal was subjected to occlusive exposure for 1hr. Just after removal, corrosion was observed adding to well defined erythema and severe edema; therefore, no more application was performed.

Based on the above results, it was concluded that was a corrosive substance under the conditions of this study.

VI. Reference

- 1) Guillot, J.P. et al.: Chemicals, ocular and cutaneous local tolerance, "cosmetic", A.F.N.O.R. and O.E.C.D. protocols, 2.2 Test for the evaluation of the cutaneous, Irritation and/or corrosivity in the rabbit, 20-25, 1982.

Table 1 Mean irritation scores in acute dermal irritation/corrosion study of in rabbits

Test substance	Number of animals	Lesion	Mean scoring ¹⁾				P.C.I.	Classification ²⁾
			1 hr.	24 hr.	48 hr.	72 hr.		
		Erythema	* (*)	* (*)	* (*)	* (*)		
	1	Edema	* (*)	* (*)	* (*)	* (*)	ND	Corrosive
		Total	* (*)	* (*)	* (*)	* (*)		

() : mean

P.C.I. : Primary Cutaneous Irritation Index.

1) : Time after removal of the closed patch

2) : Association Francaise de Normalization

* : Not scored as corrosion was noted just after removal of the closed patch.

ND: Not detected

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Table 2 Clinical signs of rabbits in acute dermal irritation/corrosion study of

Animal number	Observation period (day)
	0
1	—
day 0 : Initiation day — : No abnormality	

Study No. H-10234

Table 3 Body weights of rabbits in acute dermal irritation/corrosion study of

Animal number	Observation period (day)
	0
1	2.13
Gain	—
Day 0 : Initiation day Unit : kg	

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Appendix 1 Individual irritation scores in acute dermal irritation/corrosion study of in rabbits

Test substance	Animal number	Lesion	Individual irritation score ¹⁾			
			1 hr.	24 hr.	48 hr.	72 hr.
	1	Erythema	*	*	*	*
		Edema	*	*	*	*
		Other remark	*	*	*	*
		Total score	*	*	*	*
		Edema	*	*	*	*
		Mean total score	*	*	*	*

¹⁾: Time after removal of the closed patch.

* : Not scored as corrosion was noted just after removal of the closed patch.

Study No. H-10234

QUALITY ASSURANCE STATEMENT

Title : An Acute Irritation/Corrosion Study of in Rabbits
Study No. : H-10234

Inspection items	Performed	Reported to	
		Study director	Management
Study protocol	Dec. 28, 2010	Dec. 28, 2010	Dec. 28, 2010
Amendment to the protocol (1)	Jan. 6, 2011	Jan. 6, 2011	Jan. 6, 2011
Animal receipt and body weight measurement at delivery	Jan. 6, 2011	Jan. 6, 2011	Jan. 6, 2011
Quarantine/acclimatization, animal health assessment and grouping	Jan. 11, 2011	Jan. 11, 2011	Jan. 11, 2011
Test substance weighing and administration (3min/1hr applications in the first administration)	Jan. 12, 2011	Jan. 12, 2011	Jan. 12, 2011
Observation of skin irritation/corrosion (3min/1hr applications in the first administration)	Jan. 12, 2011	Jan. 12, 2011	Jan. 12, 2011
Raw data	Jan. 24-25, 2011	Jan. 25, 2011	Jan. 25, 2011
Final report (draft)	Jan. 24-25, 2011	Jan. 25, 2011	Jan. 25, 2011
(re-inspection)	Jan. 31, 2011	Jan. 31, 2011	Jan. 31, 2011
(final)	Feb. 5, 2011	Feb. 5, 2011	Feb. 5, 2011

Based on the above inspections, I have certified that this study was conducted in accordance with the GLP, protocol and standard operating procedures prescribed by Nippon Experimental Medical Research Institute Co., Ltd., and that this report precisely describes the methods and procedures employed in this study, and accurately reflects the raw data obtained by the conduct of the study.

Quality Assurance Unit Manager : Miki Wakabayashi <Impression of seal>

Date: February 5, 2011

Nippon Experimental Medical Research Institute Co., Ltd